Environmental Health Perspectives
Vol. 16, pp. 55-60, 1976

The Pulmonary Macrophage

by Drummond H. Bowden*

An overview of the pulmonary macrophage is provided, with particular emphasis on the origin of this cell and the adaptive mechanisms whereby the macrophagic system is able to respond to increased inhalant loads of organic and inorganic pollutants. Evidence is presented which favors an hematopoietic origin for the alveolar macrophage with a monocytic transportation compartment in the blood and an interstitial cell compartment in the lung in which cellular division and maturation may occur. Through the simple mechanism of increased cellular turnover this system of mononuclear phagocytes rapidly adapts to most inhalant challenges. In addition to its primary tasks as phagocyte and destroyer of microorganisms the macrophage plays a pivotal role in the genesis of silicotic fibrosis, and it is possible that similar mechanisms may hold for a variety of cryptogenic fibroses. Paradoxically, destruction of collagen by the dual mechanisms of phagocytosis and the secretion of lytic enzymes may also occur. The relevance of this secretory function of the macrophage to the pathogenesis of destructive diseases of the lung such as emphysema remains to be determined.

A title as general as "The Pulmonary Macrophage" allows considerable latitude of approach. Since the topic has been reviewed recently (*1,2*), I will limit my remarks to an overview of the subject as seen by a pathologist and try to pose some of the many questions that remain unanswered.

The macrophagic component of pulmonary clearance has an impressive adaptive capacity that can cope with very large particulate and aerosol loads. On occasion, however, either by virtue of an excessive load or the toxic nature of the particles, the macrophages may be overwhelmed. Such breaches of the defenses are seen in the black lung of the coal miner, farmer's lung, and the nodular fibrosis induced by the inhalation of silica.

I propose to analyse some of these parameters and to examine the proposition that the macrophage itself may initiate, or at least participate in, the genesis of certain destructive and fibrotic diseases of the lung. In this context we will look at the macrophage and its interrelationships with other cells.

*Department of Pathology, University of Manitoba, Winnipeg R3E OW3, Canada.

Origin of the Pulmonary Macrophage

The origin and mode of replication of the pulmonary macrophage is of more than academic interest. The macrophage is a phagocytic cell, and in order to be effective as an alveolar scavenger it would be a distinct advantage if it were capable of rapid adaptive proliferation in response to an unusual load of inhaled particulates.

Mononuclear phagocytes are widely distributed throughout the body, and there is good evidence supporting the concept that these cells constitute a discrete, though multisystemic, macrophagic complex. Although the ultimate precursor of the macrophage is unknown, the work of Van Furth indicates that promonocytes divide in the bone marrow to give rise to monocytes that enter the blood stream (*3*). In turn, these cells subsequently leave the circulation at random, to become tissue macrophages.

The hematopoietic origin of the alveolar macrophage is firmly established, but there has been some doubt and controversy about the final steps in the delivery of a functioning macrophage to the alveolus. Certain biochemical differences between alveolar macrophages and monocytes and

macrophages from other sites suggest that direct migration from the blood stream may not be the usual mode of entry into the alveoli (*4*). It has been proposed that blood monocytes do not migrate directly into the air sacs but pass into an interstitial compartment where they undergo division and develop the biochemical characteristics that distinguish the alveolar macrophage from other mononuclear phagocytes (*5*).

This concept of a compartment of interstitial cells which are the immediate antecedents of the free alveolar phagocytes is supported by the response of interstitial cells to a variety of natural and experimental stimuli. In some viral pneumonias, proliferation of interstitial cells is the hallmark of infection, and in sarcoidosis, silicosis, and experimentally, after the injection of Freund's adjuvant, proliferation of interstitial cells is followed rapidly by an outpouring of free alveolar macrophages.

The hypothesis has been tested by studying macrophage kinetics in cultured lung explants, a system that is free of circulating monocytes (*6*). After an initial 24-hr period of quiescence, proliferation of interstitial cells was observed, followed within 24 hr by the appearance of a new population of free alveolar macrophages.

Recently we have used the same system to determine whether these free macrophages undergo mitosis or whether their ongoing production is dependent upon the continuing division of the interstitial cells. The results suggest that a continuing supply of alveolar macrophages is dependent upon division and migration of interstitial cells rather than the mitotic activity of the free macrophagic population (*7*).

The available evidence suggests, therefore, that the free alveolar macrophage usually exists in the inactive Go phase of the mitotic cycle, an end of the line cell, destined to phagocytize and to be eliminated through the tracheobronchial tree. There may be exceptions, however. In culture, macrophages may be stimulated to enter the mitotic cycle by the addition of growth factors derived from cultures of proliferating fibroblasts (*8*). Also of some importance are two recent reports to the effect that proliferation of macrophages in culture may be induced by the addition of cell free protein rich inflammatory exudates (*9,10*). So, we may have an additional adaptive mechanism whereby an inflammatory response to alveolar injury may induce *in situ* proliferation of free alveolar macrophages.

Adaptive Responses of the Alveolar Macrophages

The concept of a total population of macrophages composed of free cells within the air sacs, their immediate antecedents in the interstitial tissues and their feeder monocytes in the peripheral blood is important to an understanding of the reactions of this cellular system to the various particulate and toxic agents that may reach the alveolar air-blood membrane.

I suggested earlier that, to be an effective alveolar phagocyte, the macrophage should be capable of rapid proliferation in response to an unusually heavy load of inhaled particulates. Brain and his colleagues have shown that the total output of free macrophages is very finely tuned to the effective load of small particles that reaches the air sacs. In a series of carefully controlled experiments (*11*) they demonstrated that macrophagic output is affected by the total load, the particle size and the chemical composition of the particle. As with most adaptive responses there is a limit, the system can be saturated. At this point, particulate matter may be observed in the cytoplasm of the type 1 epithelial cells of the alveolus and later within the interstitial tissue (Fig. 1). This may explain how under conditions of heavy and continued exposure, as in a coal mine, the dust is not completely eliminated and accumulates in the interstitium of the lung.

It has been known for many years that the fibrogenic response in the pneumoconioses is related to total load, particle size, and the physicochemical state of the respired dust. It now appears that the variable reactions obtained with different dusts may be explained, at least in part, by the initial response of the alveolar macrophage to the material it has ingested. Silica, in particular, is extremely cytotoxic to the macrophage, and it seems likely that this macrophagic reaction is a critical step in the sequential development of silicotic fibrosis of the lung. There are large gaps in our knowledge of this sequence, but we do know something about the initial changes that occur.

When inert particles or bacteria are phagocytized, the lysosomal enzymes are discharged into the phagosome and remain sequestered from the cytoplasm. When silica is ingested, lysosomes rupture, and the enzymes are released into the cytoplasm (*12*). With death of the macrophage the silica particles are released from the cell. The toxicity of these particles is, however, completely

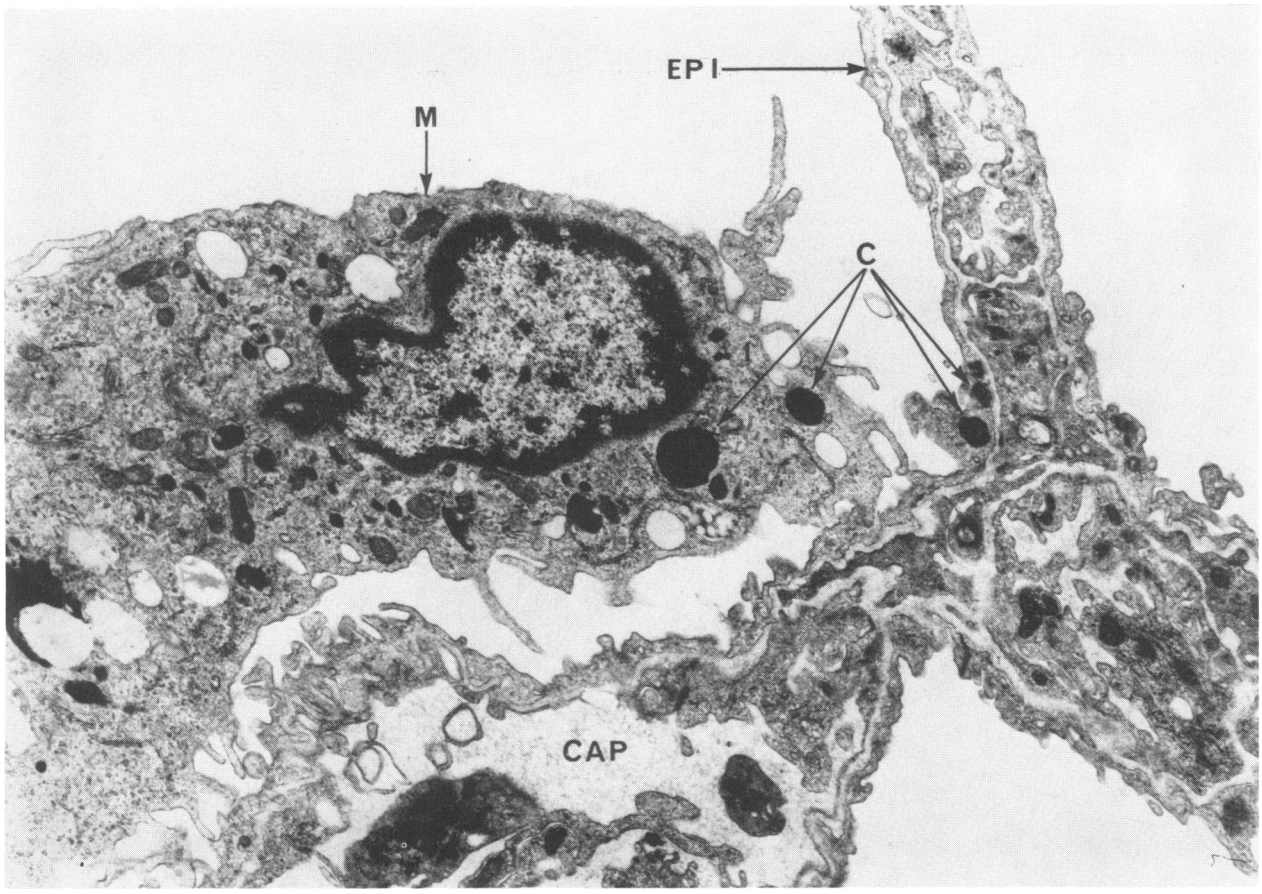

FIGURE 1. Pulmonary reaction to heavy load of inhaled carbon (C). Many particles have been phagocytized by the alveolar macrophage; some are seen within the cytoplasm of the type 1 epithelium (EPI) enroute to the interstitium. Electron micrograph × 14,000.

unaltered; this probably accounts for the repeated cycles of phagocytosis, macrophage killing and release of free silica that characterize industrial silicosis. The continuing release of silica into the air sacs also increases the likelihood of particles penetrating the air blood barrier and gaining access to the interstitium of the lung, where they provoke a collagenous response (Fig. 2).

Now while it has been relatively easy to establish a pivotal role for the macrophage in the initiation of the silicotic process, the relationship of these early events to subsequent fibrogenesis has proven to be a most difficult problem to unravel. The fibrogenic potential of a particular dust closely matches its cytotoxicity *in vitro*, and Heppleston has proposed (*13*) that the dying macrophage releases a factor which stimulates the production of collagen. This hypothesis, based upon the finding that crystalline silica stimulated the production of hydroxyproline in cultured fibroblasts has been challenged by Harington (*14*), who was unable to duplicate Heppleston's results. A recent paper by Nourse (*15*) attributes the discrepant results to differences in the respective culture systems. So, for the moment, the matter is unsettled.

Should such a principle be synthesized by the macrophage it would certainly not be the only factor involved in the development of silicosis. Heppleston has shown that specific pathogen-free animals respond differently to prolonged exposure to silica (*16*). The initial macrophagic response is observed but in these animals fibrogenesis is, in some way, inhibited. It appears that the microbial milieu of the lung is also important; here, one is reminded of the clinical evidence linking anthracosilicosis to pulmonary tuberculosis and the experimental data showing that usually nonfibrogenic dusts may induce massive pulmonary fibrosis if they are administered together

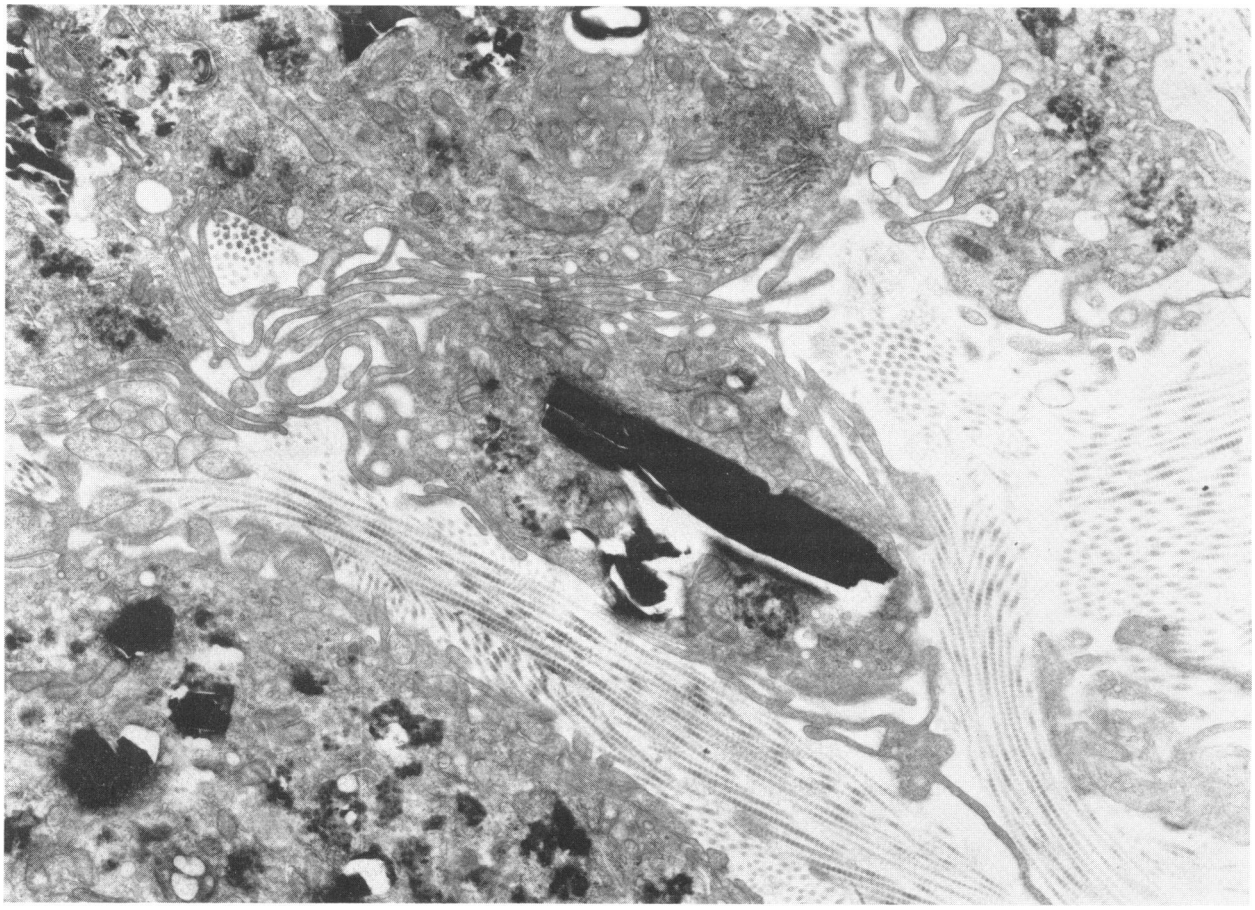

FIGURE 2. Cellular constituents of a silicotic nodule. A spicule of siliceous material lies within the cytoplasm of a macrophage which is surrounded by collagen fibers. Electron micrograph × 14,000

with avirulent strains of tubercle bacilli (*17*). Clearly, we are dealing with a highly complex situation, a situation that goes far beyond the simple hypothesis of a fibrogenic principle synthesized by the alveolar macrophage.

Whatever the mechanism, undoubtedly the macrophage plays a pivotal role in the genesis of silicotic fibrosis. As an extension to this hypothesis, Spector has suggested (*18*) that fibroblastic stimulation by injured macrophages may explain collagen deposition in a variety of the so-called cryptogenic fibroses of the lung. He also postulates a role for macrophages in the lysis of collagen and the remodelling of fibrotic scars. This idea is based upon the knowledge that, whereas the quiescent macrophage is concerned primarily with endogenous digestion, the activated macrophage is capable of exogenous enzymatic secretion (*19*). Proof of the postulate is not at hand, and at present the evidence is conflicting; in particular, the presumptive duality of the fibroblast as a collagen-secreting and a collagen-degrading cell may be mentioned (*20*). The problem awaits a definitive experiment in which the functions of cells in mixed macrophage-fibroblast populations are clearly demarcated.

There is a similar dilemma when we consider a possible role for the alveolar macrophage in the pathogenesis of destructive diseases of the lung such as emphysema. In this disease there is a progressive loss of pulmonary elasticity with consequent increase of lung compliance. Similar destructive lesions may be induced experimentally by the infusion of proteolytic enzymes into the lung (*21*). The alveolar macrophage contains proteolytic enzymes and it is tempting to postulate that release of these enzymes may provide an intrinsic mechanism of pulmonary autodigestion (Fig. 3).

The hydrolytic enzymes of the macrophage are

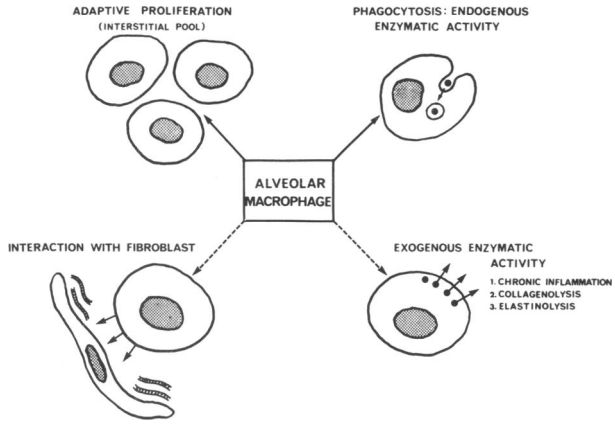

FIGURE 3. Established and postulated functions of the pulmonary macrophage.

neatly packaged in the lysosomal bodies. Extracellular levels of acid hydrolases rarely exceed 10-15% of the total activity; when the cells are stimulated, however, this percentage is increased (*19*). The relevance of this observation to the pathogenesis of emphysema must be regarded as tentative since the data are scanty and somewhat conflicting. There are pointers of interest however.

A few years ago, Janoff and his colleagues (*22*) reported low levels of an elastaselike esterase in human alveolar macrophages obtained at autopsy. More recently, Harris (*23*) demonstrated much higher enzymatic activity in fresh macrophages obtained by lavage from health volunteers. Perhaps of more importance is his finding of greatly elevated levels of activity of protease and elastase in macrophages lavaged from the lungs of smokers. If we take into account the 4-5-fold increase in the number of free alveolar macrophages in the lungs of smokers, the total increase of enzyme activity in smokers' macrophages is about 10× for esterase and 18× for the protease. In view of the recognized association between smoking and emphysema, one wonders whether this increase of endogenous enzymatic activity in smokers may be one of the factors involved in the pathogenesis of emphysema.

Whether these macrophagic enzymes are capable of digesting the tough collagen and elastic fibers of the lung *in vivo* is not known but, for the moment, the old Scottish verdict of "not proven" is appropriate.

Conclusion

The functional reserve of the pulmonary macrophage system with its pool of interstitial cells and a continuing supply of feeder monocytes in the pulmonary circulation is, through the simple mechanism of increased cellular turnover, more than adequate to handle most inhalant loads. Pathologic changes supervene only when the adaptive capacity is overwhelmed. This may occur with a massive inhalant load as experienced by the farmer in his dusty barn or by the inhalation of toxic particles of gases that injure the macrophages.

The complex interplay of the macrophage with the diverse agents that have been implicated in the pathogenesis of chronic fibrosing and destructive diseases of the lung remains to be unraveled.

REFERENCES

1. Bowden, D. H. The alveolar macrophage. Current Topics Pathol. 55:1 (1971).
2. Bowden, D. H. The alveolar macrophage and its role in toxicology. Crit. Rev. Toxicol. 2: 95 (1973).
3. Van Furth, R. The origin and turnover of promonocytes, monocytes and macrophages in normal mice. In: Mononuclear Phagocytes, R. Van Furth, Ed., Blackwell Scientific Publications, Oxford, England, 1970, p. 151.
4. Oren, R., et al. Metabolic patterns in three types of phagocytizing cells. J. Cell Biol. 17: 487 (1963).
5. Bowden, D. H., et al. Origin of the lung macrophage: evidence derived from radiation injury. Arch. Pathol. 88: 540 (1969).
6. Bowden, D. H., and Adamson, I.Y.R. The pulmonary interstitial cell as immediate precursor of the alveolar macrophage. Am. J. Pathol. 68: 521 (1972).
7. Adamson, I.Y.R., and Bowden, D. H. Alveolar macrophage kinetics in cultured explants of mouse lung. Fed. Proc. 34: 857 (1975).
8. Virolainen, M., and Defendi, V. Dependence of macrophage growth in vitro upon interaction with other cell types. Wistar Inst. Symp. Monograph 7: 67 (1967).
9. Wynne, K. M., Spector, W. G., and Willoughby, D. A. Macrophage proliferation *in vitro* induced by exudates. Nature 253: 636 (1975).
10. Adolphe, M., et al. Induction of DNA synthesis in rat macrophages *in vitro* by inflammatory exudate. Nature 253: 637 (1975).
11. Brain, J. D. The effects of increased particles on the number of alveolar macrophages. In: Inhaled Particles. III. W. H. Walton, Ed., Unwin Bros., Surrey, England, 1971, p. 209.
12. Allison, A. C., Harington, J. S., and Birbeck, M. An examination of the cytotoxic effects of silica on macrophages. J. Exptl. Med. 124: 141 (1966).
13. Heppleston, A. G., and Styles, J. A.: Activity of a macrophage factor in collagen formation by silica. Nature 214: 521 (1967).
14. Harington, J. S., et al. The *in vitro* effects of silica-treated hamster macrophages on collagen production by hamster fibroblasts. J. Pathol. 109: 21 (1973).
15. Nourse, L. D., et al. The effects of macrophages isolated from the lungs of guinea pigs dusted with silica on collagen biosynthesis by guinea pig fibroblasts in cell culture. Environ. Res. 9: 115 (1975).
16. Heppleston, A. G., and Young, A. E. Alveolar lipo-proteinosis: an ultrastructural comparison of the experimental and human forms. J. Pathol. 107: 107 (1972).

17. Zaidi, S. H., et al. Experimental infective pneumoconiosis. IV. Massive pulmonary fibrosis produced by coal mine dust and isoniazid resistant tubercle bacilli of low virulence. Brit. J. Exptl. Pathol. 36: 553 (1955).
18. Spector, W. G. Pulmonary fibrosis due to chemicals and particles. Ann. N. Y. Acad. Sci. 221: 309 (1974).
19. Cohn, Z. A. Macrophage physiology. Fed. Proc. 34: 1725 (1975).
20. Perez-Tamayo, R. Collagen resorption in carrageenin granulomas. II. Ultrastructure of collagen resorption. Lab. Invest. 22: 142 (1970).
21. Weinbaum, G., et al. Enzyme production of experimental emphysema in the dog. Route of exposure. Am. Rev. Resp. Dis. 109: 351 (1974).
22. Janoff, A., Rosenberg, R., and Goldston, M. Elastase-like esteroprotease activity in human and rabbit alveolar macrophage granules. Proc. Soc. Exptl. Biol. Med. 136: 1054 (1971).
23. Harris, J. O., et al. Comparison of proteolytic enzyme activity in pulmonary alveolar macrophages and blood leukocytes in smokers and nonsmokers. Am. Rev. Resp. Dis. 111: 579 (1975).